ACCOMPANYING DOCUMENTS/MATERIALS

Accompanying this response and preliminary amendment are the following documents and/or materials: **(1)** a Petition for Extension of Time and the fee therefor; **(2)** a Fee Transmittal; **(3)** a fully executed Combined Declaration and Power of Attorney; **(4)** substitute formal drawings (Figures 1-14) submitted in compliance with 37 C.F.R. §1.84; **(5)** a diskette containing a computer readable form (CRF) of the Sequence Listing; and **(6)** Part 2 of the Notice to File Missing Parts of Nonprovisional Application.

AMENDMENT

In the Specification:

Under the section heading entitled "Sequence Listing" please delete the entire section and replace the same with the clean replacement section entitled "Sequence Listing," consisting of 26 pages numbered "i" through "xxv,i" which replacement section is attached to this Response/Preliminary Amendment.

adult and newborn individuals are intended to be covered. The methods described herein are intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

General Overview

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

The present invention provides novel compositions containing nucleic acid sequences, wherein a first sequence in the composition is a coding sequence for an A subunit obtained or derived from an ADP-ribosylating bacterial toxin, and a second sequence in the composition is a coding sequence for a B subunit obtained or derived from an ADP-ribosylating bacterial toxin. The first and second sequences are useful in immunization methods wherein they are delivered to a subject in order to provide for an adjuvant effect (against a co-administered antigen of interest) in the immunized subject. ADP-ribosylating bacterial toxins are a family of related bacterial exotoxins and include diphtheria toxin (DT), pertussis toxin (PT), cholera toxin (CT), the *E. coli* heat-labile toxins (LT1 and LT2), *Pseudomonas* endotoxin A, *Pseudomonas* exotoxin S, *B. cereus* exoenzyme, *B. sphaericus* toxin, *C. botulinum* C2 and C3 toxins, *C. limosum* exoenzyme, as well as toxins from *C. perfringens*, *C. spiriforma* and *C. difficile*, *Staphylococcus aureus* EDIN, and ADP-ribosylating bacterial toxin mutants such as CRM_{197}, a non-toxic diphtheria toxin mutant (see, e.g., Bixler et al. (1989) *Adv. Exp. Med. Biol.* 251:175; and Constantino et al. (1992) *Vaccine*). Most ADP-ribosylating bacterial toxins are organized as an A:B multimer, wherein the A subunit contains the ADP-ribosyltransferase activity, and the B subunit acts as the binding moiety. Preferred ADP-ribosylating bacterial toxins for use in the compositions of the present invention include cholera toxin and the *E. coli* heat-labile toxins.

These and other objects, aspects, embodiments and advantages of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Drawings

Figure 1 is a restriction map and functional map of plasmid pPJV2002 that contains a truncated coding sequence for a Cholera Toxin (CT) subunit A (CTA) peptide, wherein the plasmid further contains the human cytomegalovirus (hCMV) immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated CTA expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 1) for the pPJV2002 plasmid.

Figure 2 is a restriction map and functional map of plasmid pPJV2003 that contains a truncated coding sequence for a Cholera Toxin (CT) subunit B (CTB) peptide, wherein the plasmid further contains the hCMV immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated CTB expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 2) for the pPJV2003 plasmid.

Figure 3 is a restriction map and functional map of plasmid pPJV2006 that contains a truncated coding sequence for a CTA peptide, wherein the truncated CTA coding sequence has been further modified to delete a C-terminal KDEL motif in the subunit peptide encoded thereby. The plasmid further contains the hCMV immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated CTA expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 3) for the pPJV2006 plasmid.

Figure 4 is a restriction map and functional map of plasmid pPJV2004

that contains a truncated coding sequence for an *E. coli* heat labile enterotoxin (LT) subunit A (LTA) peptide, wherein the plasmid further contains the hCMV immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated LTA expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 4) for the pPJV2004 plasmid.

Figure 5 is a restriction map and functional map of plasmid pPJV2005 that contains a truncated coding sequence for an LT subunit B (LTB) peptide, wherein the plasmid further contains the hCMV immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated LTB expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 5) for the pPJV2005 plasmid.

Figure 6 is a restriction map and functional map of plasmid pPJV2007 that contains a truncated coding sequence for an LTA peptide, wherein the truncated LTA coding sequence has been further modified to delete a C-terminal RDEL motif in the subunit peptide encoded thereby. The plasmid further contains the hCMV immediate early promoter and associated intron A sequence, and the coding sequence for the signal peptide of human tissue plasminogen activator to allow for secretion from mammalian cells of the truncated LTA expression product. The figure further contains the complete nucleic acid sequence (SEQ ID NO: 6) for the pPJV2007 plasmid.

Figure 7 depicts the results from the ELISA carried out in Example 5. The histogram represents the log reciprocal titer of anti-gp120 antibody present in the animals receiving, from left to right in the figure, Formulation #1 containing the empty pWRG7054 vector ("EmpVec"); Formulation #2 containing the EmpVec (pWRG7054) and the pCIA-EnvT plasmid ("gp120"); Formulation #3 containing the EmpVec (pWRG7054) combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B"); Formulation #4 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2002 and pPJV2003 adjuvant

vectors ("CTA/B"); Formulation #5 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2006 and pPJV2003 adjuvant vectors ("CTA-KDEL/B"); or no vaccine and/or adjuvant composition ("naive").

Figure 8 depicts the results from the *in situ* ELISA carried out in Example 5. The histogram represents the relative level of gp120-specific IFN-γ production in splenocytes obtained from animals receiving, from left to right in the figure, Formulation #1 containing the empty pWRG7054 vector ("EmpVec"); Formulation #2 containing the EmpVec (pWRG7054) and the pCIA-EnvT plasmid ("gp120"); Formulation #3 containing the EmpVec (pWRG7054) combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B"); Formulation #4 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B"); or Formulation #5 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2006 and pPJV2003 adjuvant vectors ("CTA-KDEL/B").

Figure 9 depicts the results from the ELISPOT assay carried out in Example 5. The histogram represents the relative levels of IFN-γ-producing splenocytes obtained from animals receiving, from left to right in the figure, Formulation #1 containing the empty pWRG7054 vector ("EmpVec"); Formulation #2 containing the EmpVec (pWRG7054) and the pCIA-EnvT plasmid ("gp120"); Formulation #3 containing the EmpVec (pWRG7054) combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B"); Formulation #4 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B"); or Formulation #5 containing the pCIA-EnvT plasmid ("gp120") combined with the pPJV2006 and pPJV2003 adjuvant vectors ("CTA-KDEL/B").

Figure 10 depicts the results from the ELISA carried out in Example 6. In this figure, the geometric mean absorbance values represent the titer of anti-HBcAg antibody present in serum samples (at four different dilutions) taken at the booster immunization (week 6 of the study) from animals receiving either Formulation #1 containing the HBcAg/HBsAg vector plasmid (pWRG7193); or

Formulation #2 containing the HBcAg/HBsAg vector plasmid (pWRG7193) combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B").

Figure 11 depicts the results from the ELISA carried out in Example 6. In this figure, the geometric mean absorbance values represent the titer of anti-HBcAg antibody present in serum samples (at four different dilutions) taken 2 weeks following the booster immunization (week 8 of the study) from animals receiving either Formulation #1 containing the HBcAg/HBsAg vector plasmid (pWRG7193); or Formulation #2 containing the HBcAg/HBsAg vector plasmid (pWRG7193) combined with the pPJV2002 and pPJV2003 adjuvant vectors ("CTA/B").

Figure 12 depicts the results from the ELISA carried out in Example 7. The histrogram represents the log IgG1::IgG2a ratios from each immunization group receiving, from left to right in the figure, Formulation #1 containing the pM2-FL plasmid ("M2")combined with the empty vector plasmid control (pWRG7054); Formulation #2 containing the pM2-FL plasmid combined with the pPJV2002 and pPJV2003 CTA/B adjuvant vectors ("M2 + CT"); or Formulation #7 containing the pM2-FL plasmid combined with the pPJV2004 and pPJV2005 LTA/B adjuvant vectors ("M2 + LT").

Figures 13A-13D depict the results from the IFN-γ and the IL4 ELISPOT assays used to assess the immune response to the Hepatitis B virus surface and core antigens in the first study of Example 8. The histograms represent the number of spots per $1X10^5$ spleen cells from the various experimental groups.

Figure 14 depicts the survival results from the HSV-2 virus challenge study carried out in Example 9.

Detailed Description of the Invention

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified molecules or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting. In addition, the practice